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The Persistence and Fate of Malathion Residues in Stored Beans (*Phaseolus vulgaris*) and Maize (*Zea mays*)

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Abstract: Two experimental models simulating the traditional storage conditions prevalent in Kenya, i.e. the open basket model and the modern wooden box model, were used to study the rate of dissipation and fate of malathion residues in maize grains and beans stored for periods of up to one year at ambient temperatures averaging 23°C. The grain samples were initially treated with 10·36 mg kg^{-1} of radiolabelled malathion dust prior to storage and portions analysed at regular intervals for malathion, malaoxon and the transformation products isomalathion, malathion α-monocarboxylic acid and malathion β-monocarboxylic acid using a combination of chromatographic, radioisotopic and mass-spectrometric techniques.

The findings showed a gradual penetration of malathion into the grains in amounts which were slightly higher in maize than in beans irrespective of the method of storage. After 51 weeks of storage, 34–60% of the initial residues persisted in all the grains. The total residual levels were slightly higher in beans than in maize irrespective of the storage methods though the persistence was a little higher in the wooden box than in the open basket. The rates of dissipation of the pesticide from the grains decreased with storage time and followed a biphasic pattern. Applying first-order reaction kinetics, the following half-lives were obtained: maize grains stored in open basket: 194 days; maize grains stored in closed wooden box: 261 days; beans stored in open basket: 259 days; beans stored in closed wooden box: 405 days. Beans stored in the wooden box had higher levels of bound residues than those sampled from the open basket. This trend was similar in maize grains although the concentrations were lower. The analysis of malathion metabolites confirmed the degradation trend of the residues.

Key words: stored grains, dissipation and fate of malathion residues, first order kinetics and half-lives

1 INTRODUCTION

Malathion (*S*-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorodithioate) is a widely used organophosphorus pesticide, due to its low persistence in the environment and high insecticidal activity.[1] It is used worldwide for the control of pests in vegetables, field crops, fruits, nuts, stored grains and on domestic animals.[2] In Kenya, malathion is mainly used to control insect pests in stored grains. It is commercially available as 20 g kg^{-1} malathion dust and applied at the rate of 500 grams of the dust per tonne of dry grains.[3] It controls pests by both contact and vapour activity.

Malathion has been the subject of many biochemical studies relating to its metabolism and toxicity.[1,4–7] Studies have also been conducted to follow its persistence and fate in soil, plants and grains.[8,9] Pure malathion has moderate toxicity (LD_{50} of 12 500 mg kg^{-1} for rats) but crude malathion and its formulations contain impurities which are far more toxic to mammals. These impurities are not only formed during commercial production but can also develop in the

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grains during storage.[2,10] The most toxic of these products is the oxidation product malaoxon which is responsible for the insecticidal activity of malathion. Additional transformation products can arise from hydrolysis, isomerization of the P–S and S–C linkages and de-ethylation giving malathion α- and β-monocarboxylic acids, *O,O*-dimethyl phosphorodithioate and isomalathion. Several methods for the extraction, purification and quantification of malathion transformation products in grains have been reported and include extraction with acetonitrile/water, filtration, liquid–liquid partitioning followed by TLC, HPLC, GC and GC-MS.[11–14]

This paper reports the behaviour and persistence of malathion applied to dry maize grains and beans stored in two different storage models simulating the traditional open basket and the closed modern box. The aim was to follow the dissipation of malathion and to identify some of its degradation products.

2 MATERIALS AND METHODS

2.1 Chemicals

[^{14}C]Malathion (*S*-(1,2-bis(ethoxycarbonyl)[1,2-^{14}C] ethyl *O,O*-dimethyl phosphorodithioate); specific activity: 24·5 mCi mmol^{-1} and radiochemical purity 98% (by TLC) was obtained from International Isotopes, Munich, Germany and malathion 20 g kg^{-1} dust was purchased from Rhone Poulenc in Nairobi. Non-labelled standards, malathion, malaoxon, malathion monocarboxylic acids and isomalathion were obtained from the Institute of Ecological .Chemistry, GSF, Munich, Germany. The organic solvents used, viz: iso-octane, ethyl acetate, *n*-hexane, methanol were all of residue analysis grade. Methanol, water and acetonitrile of HPLC grade were used for HPLC analysis and 'Permafluor'® and 'Hydroluma'® scintillation cocktails were used for bound and aqueous residues, respectively. All these solvents were obtained from the Institute of Ecological Chemistry, GSF, Munich. Crystalline PPO and POPOP used in liquid scintillation spectrometry were purchased from Sigma Chemical Company, UK.

2.2 Apparatus

A Waring blender was used for sample homogenization and all samples awaiting analysis were stored at −4°C in the laboratory. Precoated silica gel 60 F 254 TLC plates, paper-lined standard glass tanks saturated with solvents: *n*-hexane + ethyl acetate (60 + 40 by volume) and a Berthold UV lamp were used in TLC analysis. A Berthold Automatic Linear Analyzer TLC Scanner was also used to scan the TLC plates.

A glass column (length: 30 cm; diameter: 1 cm) plugged with glass wool and filled to 20 cm with Florisil (60–100 mesh ASTM) was used for clean-up of internal extracts while a Hewlett Packard GC model 437 fitted with a Hewlett Packard 3385 Automatic System Integrator and a capillary column HP-5 crosslinked with 5% phenyl methyl silicone (25 m × 0·2 mm × 0·5 μm film thickness) available at the Institute of Ecological Chemistry, GSF, Munich, Germany was used. Other instruments used were a Hewlett Packard HPLC with ODS column, a Finnigan ITS 40 GC-MS and a Packard 306 Automatic Sample Oxidizer, a Berthold BF 8000 Liquid Scintillation Spectrometer, and a Beckman LS6800 Scintillation Spectrometer (available at ILRAD, Nairobi).

2.3 Sample treatment and sampling procedure

Two types of storage models were used, viz. the traditional open basket (cylindrical basket: height 11 cm, diameter 25 cm) made of straw was bought from a local market and a modern wooden box (24·5 × 24·5 × 24·5 cm) was made in the Science Workshop of the University of Nairobi. Fresh beans (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) grains were obtained from a rural farm in Ahero where chemicals had not been applied. They were air-dried in the laboratory for five days. Seven hundred and fifty grams of each dry material was treated with a mixture of 12·5 μCi of [^{14}C]malathion and 0·38 g of malathion 20 g kg^{-1} dust, equivalent to 10·36 mg kg^{-1} of the pesticide. The grains were thoroughly mixed in a polythene bag, transferred to the appropriate storage model in the laboratory and stored at 20–24°C for 12 months. Time zero recovery was 6·2–7·6 mg kg^{-1} (60–73%), principal losses probably resulting from inadequate rinsing.

Sampling and analysis started 6 h after application then continued after two weeks, 1, 2, 3, 4, 6, 9 and 12 months. Each time, grain samples (25 g) were taken in triplicate from each model, washed with distilled water (3 × 30 ml) and the washings retained. The water extracts were analysed for surface residues by mixing 1 ml with 8 ml of Hydroluma cocktail and counting. The washed samples were air-dried overnight, homogenized in a Waring blender and Soxhlet extracted with methanol (150 ml) for 4 h. The extract was concentrated to 10 ml in a rotary evaporator before adding 1 ml to 8 ml of scintillator for counting. The water phases were partitioned with *n*-hexane (3 × 30 ml) and the combined organic layer concentrated to 10 ml before determination of metabolites by TLC and GC.

2.4 Analytical methods

One-gram samples of the extracted grain were combusted in a Packard 306 Oxidizer and the [^{14}C]carbon

dioxide produced trapped in Carbosorb®, mixed with Permafluor® and counted for determination of bound residues. Precoated silica gel plates were used to identify some of the metabolites in both the water extracts (surface residues) and the methanol extracts. Development was done in paper-lined standard glass tanks using the solvent system *n*-hexane + ethyl acetate (60 + 40 by volume), and the metabolites were viewed at 254 nm. TLC scanning was used to confirm the presence of [^{14}C]malathion residues.

The analytical method followed for LC, GC, HPLC and GC-MS was a modification of that reported by Cotham *et al.*[15] The methanol extract was evaporated to dryness and redissolved in ethyl acetate (5 ml) and cleaned up on a Florisil column by elution with ethyl acetate + isooctane (15 + 85 by volume; 75 ml). The eluate was concentrated and then evaporated to 2 ml under nitrogen. A sample of eluate (1 μl) was injected into the GC for analysis with the following conditions: temperature: 80°C for 1 min, then 20°C min^{-1} to 260°C, hold for 20 min; detector: FID at 300°C; injector temperature: 250°C; carrier gas: nitrogen at 1·5 ml min^{-1}. For recovery, 10 ml of 200 mg litre^{-1} of standard malathion and malaoxon in isooctane were added to the top of the Florisil column and eluted with ethyl acetate + isooctane (15 + 85 by volume; 75 ml) and concentrated as described above before injection into the GC. By comparing their peak areas with those of known standard peaks obtained by direct injection, a recovery of about 77% was achieved. All the methanol extracts were cleaned up prior to GC analysis while the *n*-hexane extracts were injected directly into the GC. Identification was by co-chromatography with known standards and quantitation by comparing peak areas with those of known calibrated standard peak areas using an automatic integrator.

The metabolite identification was further achieved by a Hewlett Packard GC-MS with EI analysis, helium gas carrier at a flow rate of 1·5 ml min^{-1} at 70 eV and similar GC conditions, and by a Hewlett Packard HPLC with an ODS column (Type: ODS 70 × 2·1 mm, 25–37 mm Co-pell ODS) and the following conditions: acetonitrile + water (70 + 30 by volume) flow rate 1·2 ml min^{-1}; injection volume 10 μl, with all samples redissolved in methanol and co-chromatographed with malathion, malaoxon, isomalathion and malathion monocarboxylic acids at 220–215 nm and 220–280 nm respectively.

3 RESULTS AND DISCUSSION

Tables 1–4 show the dissipation of malathion residues from stored grains. By combining all these data plots of percentage dissipation against storage period were obtained as shown in Figs 1–4. The dissipation curves approximate first-order kinetics with a rapid fast phase of disappearance followed by a slower second phase. By linear regression analysis, the first-order log plots (2·303

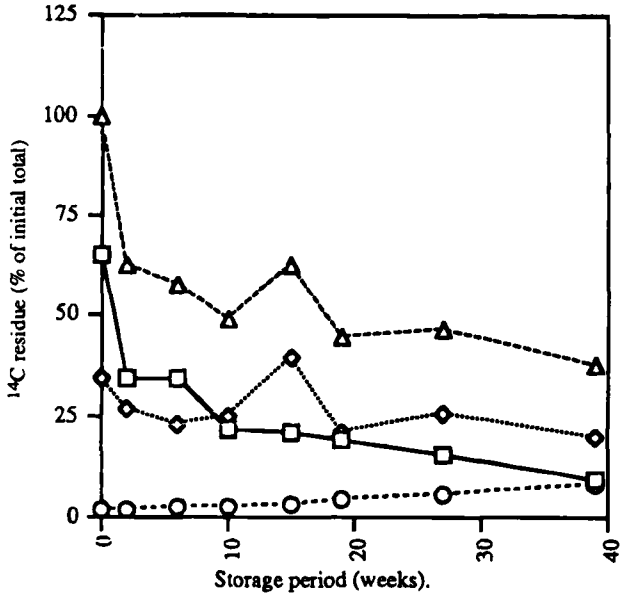

Fig. 1. Persistence of total ^{14}C residues in maize stored in open basket: (—□—) External (surface) residues; (···◇···) Internal (extractable) residues; (---○---) Bound residues; (---△---) Total (surface + extractable + bound) residues.

TABLE 1
Total ^{14}C Residues Recovered from Maize Grains Dosed with [^{14}C]malathion and Stored in the Open Basket

Storage period (*weeks*)	*External (surface) residues*		*Internal (extractable) residues*		*Bound residues*		*Total residues*	
	(*mg kg*$^{-1}$) (±*SD*)	(%)	(*mg kg*$^{-1}$) (±*SD*)	(%)	(*mg kg*$^{-1}$) (±*SD*)	(%)	(*mg kg*$^{-1}$)	(%)
0	4·92 (±0·14)	64·5	2·61 (±0·04)	34·2	0·10(±0·004)	1·3	7·62	100
2	2·61 (±0·07)	34·2	2·00 (±0·06)	26·3	0·11 (±0·002)	1·4	4·72	61·9
6	2·43 (±0·11)	33·8	1·76 (±0·02)	23·1	0·19 (±0·014)	2·5	4·39	57·5
10	1·64 (±0·03)	21·5	1·88 (±0·08)	24·7	0·19 (±0·015)	2·5	3·72	48·7
15	1·58 (±0·03)	20·7	2·98 (±0·12)	39·0	0·20 (±0·005)	2·6	4·76	62·3
19	1·46 (±0·07)	19·1	1·58 (±0·04)	20·7	0·33 (±0·008)	4·3	3·37	44·1
27	1·15 (±0·06)	15·1	1·94 (±0·07)	25·5	0·43 (±0·017)	5·6	3·52	46·2
39	0·67 (±0·03)	8·8	1·52 (±0·10)	19·9	0·62 (±0·030)	8·1	2·81	36·9

TABLE 2
Total ^{14}C Residues Recovered from Maize Grains Dosed with [^{14}C]malathion and Stored in the Wooden Box

Storage Period (weeks)	External surface) residues		Internal (extractable) residues		Bound residues		Total residues	
	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1})	(%)
0	4·62 (±0·39)	61·9	2·79 (±0·39)	37·5	0·01 (±0·004)	0·7	7·46	100
2	2·43 (±0·02)	32·6	2·07 (±0·33)	27·7	0·02 (±0·003)	1·8	4·63	62·1
6	2·25 (±0·02)	30·1	1·58 (±0·08)	21·2	0·29 (±0·004)	3·8	4·11	55·1
10	1·64 (±0·02)	22·0	2·19 (±0·02)	29·3	0·21 (±0·003)	2·9	4·04	54·2
15	1·94 (±0·02)	26·1	3·04 (±0·35)	40·7	0·16 (±0·007)	2·1	5·14	68·9
19	1·40 (±0·01)	18·7	1·64 (±0·05)	22·0	0·18 (±0·009)	2·4	3·21	43·1
27	1·21 (±0·03)	16·3	1·70 (±0·10)	22·8	0·50 (±0·052)	6·7	3·41	45·8
39	1·09 (±0·04)	14·7	2·13 (±0·13)	30·9	0·48 (±0·051)	6·4	3·88	52·0

log(% dissipation)) against time gave the calculated dissipation constants and half-lives shown in Table 5.

The dissipation results showed that high percentage levels of initial residues were found in the methanol extracts after only 6 h storage. This was about 34% in maize stored in the open basket, 37% in maize in the closed wooden box, 9% in beans in the open basket and 16% in beans stored in the closed wooden box. This implies that washing the treated seeds with water did not remove all the pesticide residues from the surface. More residues adhered the surface of maize than onto beans. These results re-emphasize the danger of toxic compounds that may be consumed with the grains/beans even after washing.

The percentage of residues on the surface decreased gradually with storage time. This decrease was from

TABLE 3
Total ^{14}C Residues Recovered from Beans Dosed with [^{14}C]malathion and Stored in Open Basket

Storage Period (weeks)	External surface) residues		Internal (extractable) residues		Bound residues		Total residues	
	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1})	(%)
0	6·64 (±0·07)	89·9	0·69 (±0·04)	9·0	0·09 (±0·004)	1·2	7·64	100
2	4·87 (±0·04)	63·6	1·05 (±0·05)	13·9	0·18 (±0·009)	2·3	6·11	80·0
6	4·40 (±0·07)	58·0	0·80 (±0·04)	10·7	0·27 (±0·034)	3·5	5·49	72·2
10	3·53 (±0·07)	46·4	1·85 (±0·03)	24·3	0·27 (±0·015)	3·5	5·67	74·2
15	3·53 (±0·03)	46·4	0·91 (±0·07)	11·9	0·53 (±0·036)	7·0	4·98	65·2
19	3·09 (±0·10)	40·6	0·80 (±0·03)	10·4	0·53 (±0·091)	7·0	4·40	58·0
27	2·00 (±0·01)	26·1	1·20 (±0·02)	15·9	1·17 (±0·01)	15·4	4·36	57·4
39	1·31 (±0·02)	17·4	0·76 (±0·02)	9·9	1·13 (±0·01)	14·8	3·2	42·0
51					1·97 (±0·10)	25·5		

TABLE 4
Total ^{14}C Residues Recovered from Beans Dosed with [^{14}C]malathion and Stored in Wooden Box

Storage Period (weeks)	External surface) residues		Internal (extractable) residues		Bound residues		Total residues	
	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1}) (±SD)	(%)	(mg kg^{-1})	(%)
0	5·09 (±0·21)	82·1	0·98 (±0·11)	16·1	0·11 (±0·06)	1·8	6·18	100
2	3·31 (±0·06)	53·6	0·91 (±0·08)	14·6	0·25 (±0·01)	3·9	4·47	72·1
6	3·09 (±0·08)	50·0	0·98 (±0·04)	16·1	0·36 (±0·01)	5·7	4·44	71·8
10	2·44 (±0·09)	39·2	0·55 (±0·03)	8·6	0·36 (±0·01)	5·7	3·31	53·6
15	2·44 (±0·02)	39·2	0·80 (±0·07)	11·4	1·13 (±0·09)	18·2	4·25	68·9
19	2·00 (±0·07)	32·1	0·55 (±0·04)	8·6	1·31 (±0·04)	21·4	3·85	62·5
27	1·78 (±0·01)	28·6	0·84 (±0·05)	13·6	0·62 (±0·01)	10·0	3·24	52·1
39	1·31 (±0·02)	21·0	0·65 (±0·07)	10·7	0·95 (±0·07)	15·0	1·71	46·7

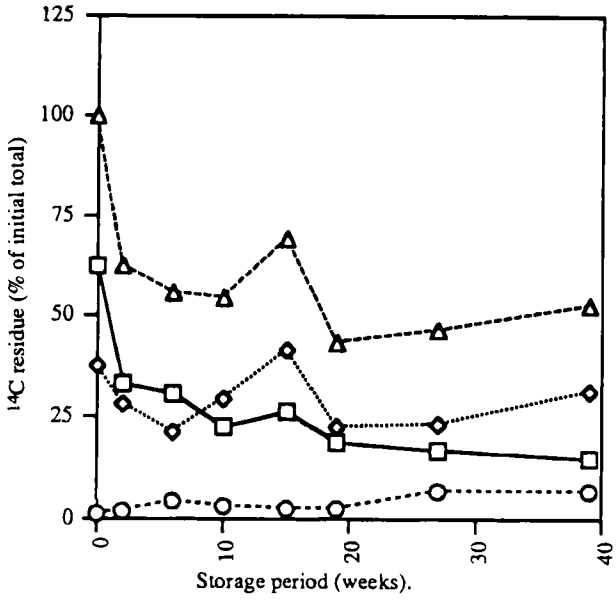

Fig. 2. Persistence of total ^{14}C residues in maize grains stored in closed wooden box: (—□—) Extractable (surface) residues2; (···◇···) Internal (extractable) residues; (---○---) Bound residues; (---△---) Total (surface + extractable + bound) residues.

64% after 6 h to 8·8% after 39 weeks for maize grains stored in the open basket, 61 to 14·6% for maize in the closed wooden box, 87 to 17% for beans stored in the open basket and 82 to 20·9% for beans in the closed wooden box. The decrease was also found to be slightly higher in maize than beans, irrespective of the method of storage, possibly as a result of higher penetration and less binding of the residues into maize grains with storage time. The dissipation trend from the methanol extracts showed some penetration of malathion residues

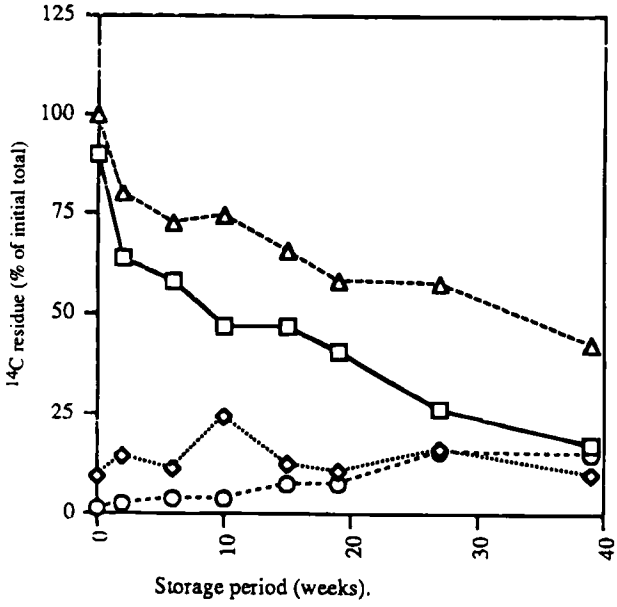

Fig. 3. Persistence of total ^{14}C residues in beans stored in open basket: (—□—) External (surface) residues; (···◇···) Internal (extractable) residues; (---○---) Bound residues; (---△---) Total (surface + extractable + bound) residues.

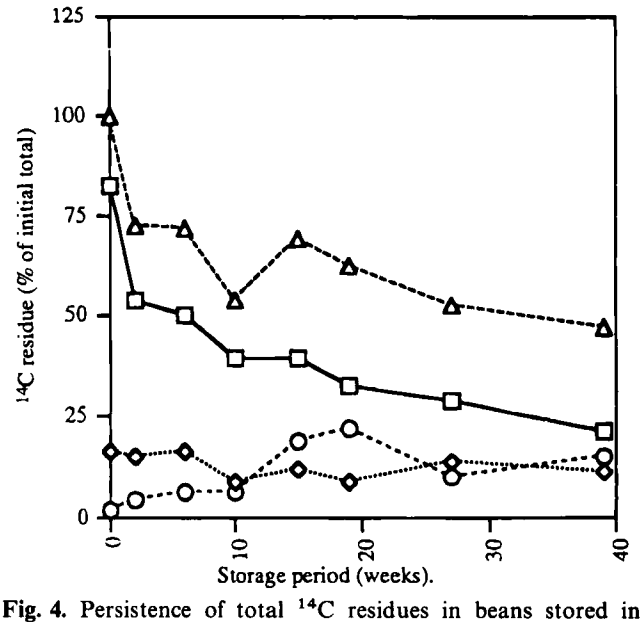

Fig. 4. Persistence of total ^{14}C residues in beans stored in closed wooden box: (—□—) External (surface) residues; (···◇···) Internal (extractable) residues; (---○---) Bound residues; (---△---) Total (surface + extractable + bound) residues.

into both maize grains and beans, particularly during the first 15 weeks and the first 10 weeks, respectively. The rate of penetration was found to be a little higher in maize than in beans.

The percentage bound residue levels increased gradually with storage time in all stored materials irrespective of the storage method. There appeared to be more binding of the pesticide in beans. The bound residue levels were higher in maize/beans in the closed wooden box than in the open basket. After 39 weeks, the bound residue levels in beans were higher i.e. 14·8% in beans stored in open basket and 15% in beans stored in the closed wooden box model. The total residues (surface + internal + bound) showed dissipation following a biphasic pattern with rapid dissipation during the first 10 weeks and a more gradual one from the tenth to the fifty-first week. In terms of total residues, the dissipation was faster from maize than from beans, irrespective of the method of storage, although dissipation was a little faster in the open basket. The dissipation curves obtained for both maize grain and beans stored under the two different storage models all approximate first-

TABLE 5
Dissipation Constants and Half-Life Values obtained by Regression Analysis

Material stored	*Dissipation constant* k *(mg kg^{-1} day^{-1})*	*Half-life (days)*
Maize grains (open basket)	−0·025	194
Maize grains (wooden box)	−0·0186	261
Beans (open basket)	−0·0187	259
Beans (wooden box)	−0·012	405

order kinetics with the dissipation constants (k) and half-lives summarized in Table 5.

Comparing the R_f values of samples spotted with those of the standards, malathion and malaoxon metabolites were identified in both the hexane (surface) and methanol extracts. TLC scanning confirmed the presence of [^{14}C]malathion in the same samples. Due to low concentrations, malaoxon and other possible metabolites could not be identified by TLC scanning. Malathion and malaoxon metabolites were readily identified by GC in both the n-hexane and the methanol extracts showing that these residues penetrated into the grains during storage.

The HPLC results indicated the presence of malathion and malaoxon in the methanol extracts of both maize and beans samples. This was achieved by filtering the methanol extracts and then directly injecting 10 μl of the filtrate into the HPLC. The malathion portion eluting from the column during analysis was collected with the help of an automatic fraction collector fitted to an HPLC radiochromatogram and its identity confirmed by GC-MS. Malathion and malaoxon were detected in the 210–215 nm UV range. The samples were concentrated and redissolved in methanol after Florisil clean-up. Isomalathion and malathion monocarboxylic acid metabolites were detected but not quantified at 222·4 nm and 280·6 nm, respectively in both beans and maize grain sampled after 19 weeks of storage. However no separation of the α- and the β-monoacids was achieved. The GC-MS results confirmed malathion and malaoxon in the n-hexane (surface) and methanol (internal) extracts samples of both beans and maize grain.

4 CONCLUSION

Malathion persists longer in beans than in maize irrespective of the method of storage. The dissipation is dependent on the type of storage and is more rapid in the open basket than in the closed wooden box. Over 50% of the pesticide had dissipated from both maize grains and beans after one year of storage. There was penetration of malathion into the seeds during storage and the formation of bound residues also increased during storage. Malathion and malaoxon metabolites were identified by GC and HPLC, and confirmed by GC-MS in the surface and internal extracts of both maize grains and beans. Isomalathion and malathion monocarboxylic acid metabolites were also detected by HPLC in the internal extracts of both grain species samples after 19 weeks of storage.

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